Sulphur Analogues of Polychlorinated Dioxins, Furans and Diphenylethers as Inducers of Aryl Hydrocarbon Hydroxylase

Kopponen, P.[A], Kärenlampi, S.[B] and Sinkkonen, S.[C]
[A]Department of Physiology, University of Kuopio, SF-70211 Kuopio, Finland
[B]Department of Biochemistry and Biotechnology, University of Kuopio
[C]Department of Chemistry, University of Jyväskylä, SF-40351 Jyväskylä, Finland

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) include several known inducers of cytochrome P450IA1. The inducing potencies of the compounds vary widely depending on their affinity to the Ah receptor which mediates the induction[1]. The most potent inducer (as the concentration is concerned) is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). There are only a few studies on analogous compounds where oxygen is replaced by sulphur. However, the presence of polychlorinated dibenzothiophenes (PCDTs) has been recently reported in incinerator gas and fly ash samples[2,3,4]. Toxicological evaluation of three PCDTs, i.e. 3,4,6,7- and 2,3,7,8-tetrachlorodibenzothiophenes (3,4,6,7-TCDT and 2,3,7,8-TCDT) as well as 1,3,4-trichlorodibenzothiophene (1,3,4-TriCDT) has been performed in Ah responsive mice[5]. PCDTs were shown to be considerably less toxic than TCDD or the most toxic PCDFs. The cytochrome P450IA1-dependent ethoxyresorufin-O-deethylase (EROD) activity increased only slightly (less than 1,5-fold) by 2,3,7,8-TCDT or 1,3,4-TriCDT. In a previous study, 2,3,7,8-tetrachlorothianthrene (2,3,7,8-TCTA) has been reported to have a high biological activity in a bioassay[6].
In the present study, three sulphur compounds were analyzed for their cytochrome P450IA1-inducing potency in a mouse hepatoma cell line Hepa-1. Hepa-1 was chosen because it possesses a highly inducible cytochrome P450IA1-dependent aryl hydrocarbon hydroxylase (AHH) activity, which is thought to play an important role in the individual risk of environmentally-caused cancer and toxicity[7]. As compared to whole animal studies, it is also a much more sensitive indicator of PCDDs, PCDFs and PCBs. Marked differences in the induction potencies were observed both among the three compounds studied and between TCDD and its sulphur analogue.

Materials and Methods

The synthesis of 2,3,7,8-TCDT[8,9], 3,3',4,4'-tetrachlorodiphenylthioether (3,3',4,4'-TCDPS)[10] and 2,3,7,8-TCTA[11] have been published elsewhere. A subclone Hepa-1c1c7 of the mouse hepatoma cell line Hepa-1 was exposed for 24 h to various concentrations of the sulphur analogues dissolved in dimethyl sulphoxide. AHH was assayed as described[12]. TCDD was used as a reference compound.

Results and Discussion

Three sulphur analogues of PCDDs, PCDFs and polychlorinated diphenylethers were studied for their potency to induce AHH activity in a mouse hepatoma cell line. Structures of the compounds are presented in Figure 1. Having viability of cells as the end point, none of the compounds showed marked toxicity at concentrations around the EC50 values for AHH induction.

The sulphur analogue of TCDD, i.e. 2,3,7,8-TCTA, was the most potent inducer of AHH (Fig. 2). The maximal induction was caused by 3 nM 2,3,7,8-TCTA and was 21-fold as compared to the control. The 2,3,7,8-TCTA concentration eliciting the half-maximal AHH induction (EC50) was around 800 pM. When these numbers are compared to those given by TCDD, it can be seen that the maximal inducing potency for both compounds is about the same. However, the EC50 value for TCDD is around 10 pM[12]. The conclusion is, therefore, that replacement of oxygen atom by sulphur greatly decreases the potency to induce AHH activity.

Another compound eliciting the induction of AHH was the sulphur analogue of 2,3,7,8-tetrachlorodibenzofuran, i.e. 2,3,7,8-TCDT (Fig. 2). The maximal AHH induction was not quite reached by even the highest concentration available for these studies. A 23-fold induction was, however, elicited by 15,6 nM 2,3,7,8-TCDT. Mäntylä et al.[5] have recently reported the induction by 2,3,7,8-TCDT of another cytochrome P450IA1-dependent activity, EROD, in mice. The fold-induction was less than 1,5, indicating a far lower sensitivity of whole mouse as compared to the mouse hepatoma cell line. In the present study, the EC50 for 2,3,7,8-TCDT was estimated to be close to or greater than 4 nM. The same rank order of potency as detected here between 2,3,7,8-TCTA and 2,3,7,8-TCDT also exists between TCDD and 2,3,7,8-TCDF[1].

The compound which did not elicit any AHH induction was the tetrachlorodiphenylthioether 3,3',4,4'-TCDPS. The only conclusion that can be drawn is thus that the induction of AHH requires a concentration higher than 780 nM 3,3',4,4'-TCDPS to be detectable. The PCB with a structure similar to 3,3',4,4'-TCDPS but without sulphur atom between the benzene rings (3,3',4,4'-tetrachlorobiphenyl) has a potency 100 times less than TCDD[1].

There was thus a fairly predictable rank order of AHH inducing potencies among the three compounds studied. It was also obvious that the replacement of oxygen by sulphur decreased the potency. There are at least two possible explanations for this. One explanation is that the sulphur atom, being greater than oxygen, causes lengthening of the chemical bonds involved. This would then change the structure of the compounds and cause steric hindrances to the binding of the compound to the Ah receptor. However, CoMFA modelling of the Ah receptor binding affinity of PCDDs and PCDFs does not support this idea (K. Tuppurainen, personal communication; see Poso et al.[13]). Assuming that this model is valid also for sulphur analogues, they should have even higher affinity to the Ah receptor than the respective oxygen-containing compounds. The other explanation relates to the fact that the charge of oxygen is slightly negative but that of sulphur is strongly positive. Depending on the charge distribution in the ligand binding site of the Ah receptor, the positive sulphur may be rejected. The sulphur analogues thus offer interesting tools for further predictions of the structure of the ligand-binding site of the Ah receptor. This, together with the fact that the ligand-binding subunit of the Ah receptor has been recently cloned[14] open up new possibilities to explore the mechanism by which some compounds induce AHH and others do not.

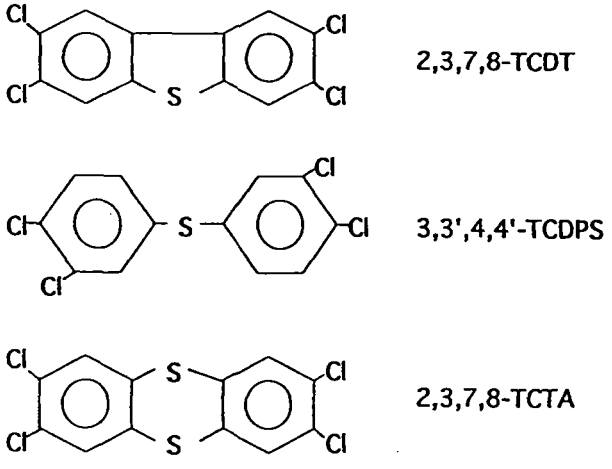

Figure 1. Structures of the compounds studied for their AHH inducing potency.

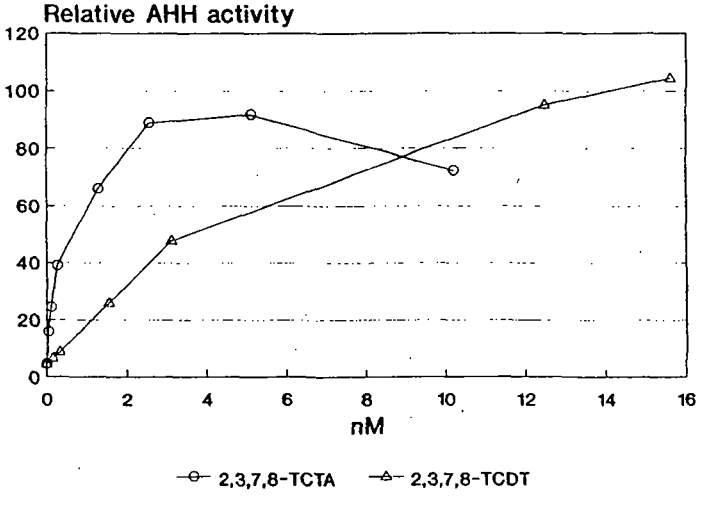

Figure 2. Dose-response curve for the induction of AHH by 2,3,7,8-TCTA and 2,3,7,8-TCDT. The induction elicited by 1 nM TCDD has been given a value of 100 and the other activities have been related to that.

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